This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 21!

See attached form for additional information.

Interagency Report Control No.:

RT

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. CERTIFICATE NUMBER: 85-R-0003
CUSTOMER NUMBER: 1072

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

Lovelace Respiratory Research Institute
2425 Ridgecrest Se
Albuquerque, NM 87108

Telephone: (505) -348-9400

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, or experimentation, or held for these purposes. Attach additional sheets if necessary)

FACILITY LOCATIONS (Sites) - See Atached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS Form 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not ye used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use o pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals an for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for wh the use of appropriate anesthetic, analgesic, or tranquiliz drugs would have adversely affected the procedures, res or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reasc such drugs were not used must be attached to this report	F. TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs	37	48	124	0	172
5. Cats	0	0	0	0	0
6. Guinea Pigs	47	0	98	0	98
7. Hamsters	0	0	0	0	0
8. Rabbits	35	18	106	56	180
9. Non-human Primates	20	0	245	187	432
10. Sheep	0	0	0	0	0
11. Pigs	0	0	0	0	0
12. Other Farm Animals	N/A 0	0	0	0	0
13. Other Animals					
Ferrets	43	12	36	259	307

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anestetic, analgesic, and tranquilizing drugs, prior to, during, and following actual rese teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and ap Institutional Animal Care and Use Committee (IACUC). **A summary of all such exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary inc brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional Official)

NOV 18 2008

SI[redacted] L (Type or Print) (b)(6), (b)(7)c DATE SIGNED 11-14-08

API

(AUG 91)

Certificate No: 85-R-0003

Category E Explanations

This Institute had 21 studies with USDA covered species in Category E during the reporting period of 10/1/07 – 9/30/08.

Study 1: 16 New Zealand White Rabbits

This study is classified as a Category E. It will look at the efficacy of a vaccine in a New Zealand white rabbit model of an infectious agent. The result of this study may be used to support future submissions to the FDA under the "Animal Rule" amendment (21 CFR Parts 314 subpart 1 and 601 subpart H) to FDA's new drug and biological products regulations. The "Animal Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the rabbits with a lethal dose of agent and follow the disease process up to death. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of infection. Also, it is not my intention as the Study Director to allow the animals to die unobserved in their cages. When the animals exhibit signs that they will not survive to the next observation period they will be humanely euthanized. This is done with full realization that validated endpoints of imminent death do not yet exist in this rabbit model. We will use out best scientific judgment, to make the call for euthanasia; however, some rabbits may not survive to the next observation period.

Study 2: 2 Nonhuman Primates (cynomolgus macaques)

The painful part of this study is the infection itself. There are no alternative to determine whether this infectious agent is exhaled other than infection of the nonhuman primate and examining what is exhaled.

The illness experienced by animals exposed to this infectious agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of infection.

Monkeys are examined by laboratory animal technicians and research technicians twice per day (morning and afternoon) on each day of the study according to SOP TXP-1532. Based on previous experience with infected monkeys, twice daily observations are adequate to monitor the progression the disease process in the animals and to minimize the duration of pain and distress prior to euthanasia. The Study Director and a staff veterinarian make decisions regarding the euthanasia of moribund animals. As part of the decision process for determining if euthanasia is appropriate, a Euthanasia Score sheet is completed each day the monkeys are weighed or more often as directed by the Study Director.

Also, it is not the Study Director's Intention to allow the animals to die unobserved in their cages. When the animals exhibit signs that they will not survive to the next observation period they will be humanely euthanized. This is done with full realization that validated endpoints of imminent death do not yet exist in this nonhuman primate disease model. We will use out best scientific judgment, to make the call for euthanasia: however, some monkeys may not survive to the next observation period.

Study 3: 2 Nonhuman Primates (cynomolgus macaques)

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Category E Explanations

This is a preliminary study to determine which factors are important in the development of certain infectious diseases. The disease process in primates instilled with mutant pathogens lacking a particular factor (lethal factor, edema factor or protective antigen) will be compared the disease process following bronchial instillation of a wild-type pathogen. If a mutant does not cause disease, then it will be eliminated from future studies to determine the ED50, but may still be considered for further pathology studies. This is a pilot study, so we need to follow the disease process without interference in order to understand the pathogenesis of the disease induced by the various strains of this pathogen.

The illness experienced by animals exposed to this organism must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production (Geher, W.F., et al, J. Toxicol Environ Health 2:577-582, 1977, Hung, C.Y. et al Proc Soc Exp Biol Med 142-106-111-1973(Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B, et al., Brain Behav Immun 3: 129-137 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G, et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C. and Marone, G, Chem Immunol 62: 108-131 1995) and Respiratory Depression (Soma, L.R. Ann NY Acad Sci 406: 32-47, 1995). Histamine is a well known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory disease by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A. et al, J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G. et al, Int Arch Allergy Immunol, 124 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois J., et.al.J. Immunol 164:2964-2970, 2000). Clearly the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333 1999) provide and additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

When the animals exhibit signs that they will not survive to the next observation period they will be humanely euthanized. This is done with full realization that validated endpoints of imminent death do not yet exist in this nonhuman primate disease model. We will use out best scientific judgment, to make the call for euthanasia: however, some monkeys may not survive to the next observation period.

Study 4: 43 Nonhuman Primates (African Green Monkeys)

This study is classified as a Category E. This is a continuation study to determine the natural history, pathogenesis of an infectious agent in the African Green Monkey. The disease process in African Green Monkeys instilled with this pathogen will be followed. We need to follow the disease process without interference in order to understand the pathogenesis of the disease induced by the pathogen in the African Green Monkeys. This is a preliminary study with the goal that this model will be used to test therapeutics or vaccines. African Green monkeys have been used to test vaccines in the past and could potentially be a useful model for development of therapeutics for this pathogen.

The illness experienced by animals exposed to this infectious agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to

Category E Explanations

interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production (Geher, W.F., et al, J. Toxicol Environ Health 2:577-582, 1977, Hung, C.Y. et al Proc Soc Exp Biol Med 142-106-111-1973(Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B, et al., Brain Behav Immun 3: 129-137 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G, et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C. and Marone, G, Chem Immunol 62: 108-131 1995) and Respiratory Depression (Soma, L.R. Ann NY Acad Sci 406: 32-47, 1995). Histamine is a well known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory disease by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A. et al, J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G. et al, Int Arch Allergy Immunol, 124 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois J., et.al.J. Immunol 164:2964-2970, 2000). Clearly the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333 1999) provide and additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

If monkey's exhibit signs that they will not survive to the next observation period they will be humanely euthanized. This is done with full realization that validated endpoints of imminent death do not yet exist in this African Green Monkey model. We will use out best scientific judgment, to make the call for euthanasia: however, some monkeys may not survive to the next observation period.

Study 5: 18 Nonhuman Primates (cynomolgus macaques)

This study is classified as a Category E. It will look at the efficacy of a vaccine against an infectious agent in the Cynomolgus monkey model. This study will support future submissions to the FDA under the "Animal Rule" amendment (21 CFR Parts 314 subpart 1 and 601 subpart H) to FDA's new drug and biological products regulations. The "Animal Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the monkeys with a lethal dose of pathogen and follow the disease process up to death. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of monkey infection. Also, it is not my intention as the study director to allow the animals to die unobserved in their cages. When the animals exhibit signs that they will not survive to the next observation period they will be humanely euthanized. This is done with full realization that validated endpoints of imminent death do not yet exist in this monkey disease model. We will use our best scientific judgment, to make the call for euthanasia; however, some monkeys may not survive to the next observation period. We will include a euthanasia score sheet that will be used to assist in determining whether an animal is moribund and not likely to live until the next observation period.

The illness experienced by animals exposed to this agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret

the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production (Geher, W.F., et al, J. Toxicol Environ Health 2:577-582, 1977, Hung, C.Y. et al Proc Soc Exp Biol Med 142-106-111-1973(Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B, et al., Brain Behav Immun 3: 129-137 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G, et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C. and Marone, G, Chem Immunol 62: 108-131 1995) and Respiratory Depression (Soma, L.R. Ann NY Acad Sci 406: 32-47, 1995). Histamine is a well known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory disease by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A. et al, J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G. et al, Int Arch Allergy Immunol, 124 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois J., et.al.J. Immunol 164:2964-2970, 2000). Clearly the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333 1999) provide and additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

Study 6: 15 Nonhuman Primates (African Green Monkeys)

This study is classified as a Category E. It will look at the efficacy of the antibiotic Levofloxacin in the African Green Monkey model of an infectious agent. This study will support future submissions to the FDA under the "Animal Rule" amendment (21 CFR Parts 314 subpart 1 and 601 subpart H) to FDA's new drug and biological products regulations. The "Animal Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the monkeys with a lethal dose of agent and follow the disease process up to death in the untreated animals in order to determine the efficacy of the antibiotic. The giving of pain or stress relieving agents is contraindicated because it may interfere in determining the efficacy of the therapeutic. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of infection. Also, it is not intended that the animals die unobserved in their cages as the telemetry allows for continuous monitoring which can be reviewed remotely. The data will be reviewed at least 3 times a day (early morning, mid-day, late afternoon) by the study director or a designee. When animals exhibit signs that they will not survive to the next observation period (i.e. when temperatures fall below 34@C, heart rates> 200 beats/min, respiratory rates of >60 breaths/min) the animal will be humanely euthanized.

The illness experienced by animals exposed to this infection must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production (Geher, W.F., et al, J. Toxicol Environ Health 2:577-582, 1977, Hung, C.Y. et al Proc Soc Exp Biol Med 142-106-111-1973(Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B, et al., Brain Behav Immun 3: 129-137 1989). Also, analgesics including

buprenorphine can cause histamine release (Marone, G, et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C. and Marone, G, Chem Immunol 62: 108-131 1995) and Respiratory Depression (Soma, L.R. Ann NY Acad Sci 406: 32-47, 1995). Histamine is a well known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory disease by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A. et al, J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G. et al, Int Arch Allergy Immunol, 124 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois J., et.al.J. Immunol 164:2964-2970, 2000). Clearly the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333 1999) provide and additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

Study 7: 60 Ferrets

The ferret model is selected for its similarities to some human infections (Renegar, 1992) and for the pathogenicity of specific infectious agents in ferrets. This proposal tests the TIGER platform (commercially referred to as the Ibis T5000) system in the ferret model of infection and provides a high level of controlled detail not available from infected humans.

The illness experienced by animals exposed to the pathogen must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production (Geher, W.F. et.al. J. Toxicol Environ Health 2:577-582, 1977: Hung, C. Y., et al. Proc Soc Exp Biol Med 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al. Brain Behav Immun 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C., and Marone, G., Chem Immunol 62: 108-131, 1995) Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has shown to induce activation of human macrophages (Mozzzoni, A., et al. J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G., et al., Int Arch Allergy Immunol, 124, 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois, J., et al., J. Immunol 164: 2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the Immune response. In this case, both opioids caused significant decrease in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

Study 8: 24 Nonhuman Primates (cynomolgus macaques)

This study is classified as a Category E. It will look at the efficacy of vaccines in the Cynomolgus macaque model of infection. This study will support future submissions to the United States Food and Drug administration (FDA) under the "Animal Efficacy Rule" amendment (21 CFR Parts 314 subpart 1 and

Category E Explanations

601 subpart H) to FDA's new drug and biological products regulations. The "Animal Efficacy Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the monkeys with a lethal dose of agent and follow the disease process up to death in the untreated animals in order to determine the efficacy of the vaccine. The giving of pain or stress relieving agents is contraindicated because it may interfere in determining the efficacy of the therapeutic. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of infection. In addition, it is not intended that the animals die unobserved in their cages as the telemetry allows for continuous monitoring which can be reviewed remotely. The data will be reviewed at least 3 times a day (early morning, mid-day, late afternoon) by the study director or a designee. When animals exhibit signs that they will not survive to the next observation period (i.e. when temperatures fall below 34@C, heart rates> 200 beats/min, respiratory rates of >60 breaths/min) the animal will be humanely euthanized.

The illness experienced by animals exposed to the pathogen must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production (Geher, W.F. et.al. J. Toxicol Environ Health 2:577-582, 1977: Hung, C. Y., et al. Proc Soc Exp Biol Med 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al. Brain Behav Immun 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C., and Marone, G., Chem Immunol 62: 108-131, 1995) Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has shown to induce activation of human macrophages (Mozzzoni, A., et al. J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G., et al., Int Arch Allergy Immunol, 124, 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois, J., et al., J. Immunol 164: 2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the Immune response. In this case, both opioids caused significant decrease in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

Study 9: 4 Nonhuman Primates (cynomolgus macaques)

This study is classified as a Category E. It will be necessary to challenge some primates with an infectious agent in order to determine the efficacy of the vaccines. The eventual goal of the project is to test vaccine candidates for submission to the FDA under the "Animal Efficacy Rule" amendment (21 CFR parts 314 subpart I and 601 subpart H) to FDA's new drug and biological products regulation. The "Animal Efficacy Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the primates with a lethal dose of pathogen and

Category E Explanations

follow the disease process up to near death.. The giving of pain or stress relieving agents is contraindicated as the progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of primate infection is necessary

The illness experienced by animals exposed to the infectious agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production (Geher, W.F. et.al. J. Toxicol Environ Health 2:577-582, 1977: Hung, C. Y., et al. Proc Soc Exp Biol Med 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al. Brain Behav Immun 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C., and Marone, G., Chem Immunol 62: 108-131, 1995) Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has shown to induce activation of human macrophages (Mozzzoni, A., et al. J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G., et al., Int Arch Allergy Immunol, 124, 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois, J., et al., J. Immunol 164: 2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the Immune response. In this case, both opioids caused significant decrease in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

When the animals exhibit signs that they will not survive to the next observation period they will be humanely euthanized. This is done with full realization that validated endpoints of imminent death do no yet exist in this primate disease model. We will use out best scientific judgment, to make the call for euthanasia; however, some NHP may not survive to the next observation period. We will use a euthanasia sheet that will be used to assist in determining whether an animal is moribund and not likely to live until the next observation period. If the vaccine is efficacious in the prevention of the disease or leads to milder form of the disease, then it will be appropriate to review the pain category at the end of the study and re-assign the animals based on the outcome of the vaccine treatments.

Study 10: 1 New Zealand White Rabbit

This animal was listed as Category E because it had an idiosyncratic adverse reaction to an injectable tranquilization agent prior to euthanasia. The animal was quickly anesthetized but did experience more than momentary distress prior to loss of consciousness. This was an unanticipated physiological response with no way to predict that the use of an agent to prevent anxiety and distress would result in pain/distress.

Study 11: 33 Nonhuman Primates (cynomolgus)

This is a preliminary study to determine which factors are important in the development of diseases associated with specific infectious agents. The disease process in primates instilled with a mutant pathogen lacking a particular factor (lethal factor, edema factor or protective antigen) will be compared

the disease process following bronchial instillation of the wild-type pathogen. If a mutant does not cause disease, then it will be eliminated from future studies to determine the ED50, but may still be considered for further pathology studies. This is a pilot study, so we need to follow the disease process without interference in order to understand the pathogenesis of the disease induced by the various strains of this agent.

The illness experienced by animals exposed to this infection must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. It is likely the use of analgesics will interfere with the accurate measurements of the disease process. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production (Geher, W.F., et al, J. Toxicol Environ Health 2:577-582, 1977, Hung, C.Y. et al Proc Soc Exp Biol Med 142-106-111-1973(Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B, et al., Brain Behav Immun 3: 129-137 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G, et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C. and Marone, G, Chem Immunol 62: 108-131 1995) and Respiratory Depression (Soma, L.R. Ann NY Acad Sci 406: 32-47, 1995). Histamine is a well known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory disease by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (Mozzoni, A. et al, J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G. et al, Int Arch Allergy Immunol, 124 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois J., et.al.J. Immunol 164:2964-2970, 2000). Clearly the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333 1999) provide and additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decreases in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

Study 12: 6 Nonhuman Primates (cynomolgus macaques)

The study is classified as a category E. The goal of the project is to challenge some nonhuman primates with an infectious agent in order to determine the 50% lethal dose (LD50) of the agent and to develop a model of the disease that can be used for future vaccine and therapeutic testing. Information collected in these experiments will provide the background necessary for development of new vaccine candidates and therapeutic agents for use in humans. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the nonhuman primates with a lethal dose of pathogen and follow the disease process to near death. Administration of pain or stress relieving agents is contraindicated as they may alter the progression of the disease process. We will use an up and down procedure for dosing in order to minimize the number of animals used.

The illness experienced by animals exposed to this pathogen must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production (Geher, W.F. et.al. J. Toxicol Environ Health 2:577-582, 1977: Hung, C. Y., et al. Proc Soc Exp Biol Med 142:106-111-1973). Moreover opioids can suppress

Category E Explanations

Natural Killer (NK) cell activity (Beilin, B., et al. Brain Behav Immun 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C., and Marone, G., Chem Immunol 62: 108-131, 1995) Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has shown to induce activation of human macrophages (Mozzzoni, A., et al. J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G., et al., Int Arch Allergy Immunol, 124, 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois, J., et al., J. Immunol 164: 2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decrease in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

When the animals exhibit signs that they will not survive to the next observation period, they will be humanely euthanized. This is done with full realization that validated endpoints of imminent death do not yet exist in this primate disease model. We will use our best scientific judgment to make the call for euthanasia: however, some nonhuman primates may not survive to the next observation period. We will use a euthanasia score sheet that will be used to assist in determining whether an animal is moribund and not likely to live until the next observation period.

Study 12: 92 Ferrets

This study is classified as a Category E. This is a study to determine the natural history, pathogenesis of an infectious agent in ferrets. The disease process in ferrets instilled with the infectious agents will be monitored. We need to follow the disease process without interference in order to understand the pathogenesis of the disease induced by this organism in ferrets. This is a preliminary study with the goal that this model will be used to test therapeutics or vaccines.

The illness experienced by animals exposed to this pathogen must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the date obtained in this study. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production. (Geher, W.F. et.al. J. Toxicol Environ Health 2:577-582, 1977: Hung, C. Y., et al. Proc Soc Exp Biol Med 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al. Brain Behav Immun 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C., and Marone, G., Chem Immunol 62: 108-131, 1995) Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has shown to induce activation of human macrophages (Mozzzoni, A., et al. J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G., et al., Int Arch Allergy Immunol, 124, 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois, J., et al., J. Immunol 164: 2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators,

using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the Immune response. In this case, both opioids caused significant decrease in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

If ferrets exhibit signs that they will not survive to the next observation period they will be humanely euthanized. This is done with full realization that validated endpoints of imminent death do not yet exist in this ferret model. We will use our best scientific judgment, to make the call for euthanasia however; some ferrets may not survive to the next observation period.

Study 13: 16 Ferrets

The objective of this study is to assess the pathogenesis of an infectious agent via different routes of administration, as well as to optimize an aerosol delivery model for the future administration of therapeutic in this animal model. Animals being challenged with this are expected to experience illness related to the infection, including fever, gastrointestinal and neurological disorders, anorexia, dehydration, hematological and clinical chemistry changes, ultimately ending in humane termination (euthanasia) prior to death. Animals being used to optimize the aerosol delivery system are not expected to experience pain of discomfort, and will be anesthetized during these procedures. Illness experienced by challenged animals must not be treated with analgesics, as this would compromise the scientific integrity of the study, mask the pathogenesis of the disease (an obscure secondary efficacy parameters, such as amelioration of clinical signs), could inadvertently accelerate the disease process (additive effect to the infection) and confound the interpretation of euthanasia criteria. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study, Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production (Geher, W.F. et al., J. Toxicol Environ Health 2:577-582, 1977 Hung, C. Y., et al. Proc Soc Exp Biol Med 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al. Brain Behav Immun 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C., and Marone, G., Chem Immunol 62: 108-131, 1995) Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has shown to induce activation of human macrophages (Mozzzoni, A., et al. J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G., et al., Int Arch Allergy Immunol, 124, 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois, J., et al., J. Immunol 164: 2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the Immune response. In this case, both opioids caused significant decrease in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).

While every attempt will be made to humanely euthanize animals that are suffering, the pathogenesis of this infection can result in rapid decline, and animals may not survive to the next observation period. This study is being conducted under the Animal Rule (subpart 1 of 21CFR314 and subpart H of 21CFR601) which allows for testing therapeutics in animals when tests can not ethically be tested in humans and field efficacies tests are not feasible.

Study 14: 24 Ferrets

Certificate No: 85-R-0003

Category E Explanations

The objective of this study is to assess the efficacy of a treatment involving the passive transfer of antibodies into ferrets prior to, or following challenge with an infectious agent. Animals receiving control material are expected to experience illness related to the infection, including fever, gastrointestinal and neurological disorders, anorexia, dehydration, hematological and clinical chemistry changes, ultimately ending in humane termination (euthanasia) prior to death. Animals receiving therapeutic are anticipated to survive and may not experience discomfort, however it cannot be ruled out that there may be subclinical pain/suffering. Illness experienced by challenged animals must not be treated with analgesics, as this would compromise the scientific integrity of the study, mask the pathogenesis of the disease (an obscure secondary efficacy parameters, such as amelioration of clinical signs), could inadvertently accelerate the disease process (additive effect to the infection), and confound the interpretation of euthanasia criteria. While every attempt will be made to humanely euthanize animals that are suffering the pathogenesis of this infection can result in rapid decline and animals may not survive to the next observation period.

Study 15: 39 New Zealand White Rabbits (revised 11/24/08)

This study is classified as a Category E. It will look at the efficacy of a vaccine in a New Zealand White rabbit model of infection. The results of this study may be used to support future submission to the FDA under the "Animal Rule" (21 CFR Parts 314 subpart 1 and 601 subpart H) amendment to the FDA's new drug and biological products regulations. The "Animal rule" applies when adequate and when clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge rabbits with a lethal dose of agent and follow the disease process up to death. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of infection. Also, it is not my intention as Study Director to allow the animals to dies unobserved in their cages. When the animals exhibit signs that they will not survive to the next observation period they will be humanely euthanized. This is done with full realization that validated endpoints of imminent death do not yet exist in this rabbit anthrax model. We will use our best scientific judgment to make the call for euthanasia; however, some rabbits may not survive to the next observation period.

Study 16: 20 Nonhuman Primates (cynomolgus macaques)

This study is classified as a Category E. This study will support future submissions to the FDA under the "Animal Rule" amendment (21 CFR Parts 314 subpart 1 and 601 subpart H) to FDA's new drug and biological products regulations. The "Animal Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the monkeys with a lethal dose of agent and follow the disease process up to death in the untreated animals in order to determine the efficacy of the vaccine. The giving of pain or stress relieving agents is contraindicated because it may interfere in determining the efficacy of the therapeutic. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of infection. Also, it is not intended that the animals die unobserved in their cages. Animals will be observed at least 2 times a day (morning and afternoon) by trained technicians. When the animals exhibit signs that they will not

survive to the next observation period the Study Director will be notified, a veterinarian will be called to confirm the diagnosis, and the animal will be humanely euthanized.

The illness experienced by animals exposed to this infectious agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the date obtained in this study. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production. (Geher, W.F. et.al. J. Toxicol Environ Health 2:577-582, 1977: Hung, C. Y., et al. Proc Soc Exp Biol Med 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al. Brain Behav Immun 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C., and Marone, G., Chem Immunol 62: 108-131, 1995) Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has shown to induce activation of human macrophages (Mozzzoni, A., et al. J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G., et al., Int Arch Allergy Immunol, 124, 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois, J., et al., J. Immunol 164: 2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the Immune response. In this case, both opioids caused significant decrease in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS)

Study 17: 10 Nonhuman Primates (rhesus macaques)

This study is classified as a Category E. The FDA "animal rule" allows for potential therapeutic and prophylactic modalities for specific toxins to be tested in animals. This is particularly so if tests cannot be performed in humans for ethical reasons. When human testing is not possible, therapeutic modalities are to be tested in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well characterized animal model for predicting the response in humans [21 CFR Part 314 Subpart 1 (New Drugs) and Part 601 Subpart H (Biological Products)]. The rhesus macaque model o has been used by many investigators and is an accepted model of toxin-induced disease for evaluation of therapeutic or prophylactic efficacy.

This is an efficacy study in a series of studies designed to support the regulatory approval and licensure of an antitoxin as a prophylactic and therapeutic biologic for the disease.

The illness from the toxin is the procedure which will cause the discomfort and distress in the animals. The illness experienced by animals exposed to the toxin must not be treated. Treatment of humans with this disease consists of supportive care (fluid and nutritional support, assisted ventilation, treatment of complications, perhaps antibiotics if secondary infections are present) and passive immunization with equine antitoxin (JAMA. 85 1059-1070, 2001). This type of treatment is not appropriate for these study animals as the goal of the project is to test the efficacy of antitoxin and other treatments may interfere with this assessment.

Study 18: 22 Ferrets

Certificate No: 85-R-0003

Category E Explanations

This is a study to determine the efficacy of a monoclonal antibody in the control and prevention of infection in ferrets. Some animals may not adequately respond to treatment with the antibody. The disease process resulting from infection will be monitored in these animals.

The illness experienced by animals exposed to this infectious agent must not be treated with analgesics because treatment will interfere with studying the pathogenesis of the disease. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the date obtained in this study. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production. (Geher, W.F. et.al. J. Toxicol Environ Health 2:577-582, 1977: Hung, C. Y., et al. Proc Soc Exp Biol Med 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al. Brain Behav Immun 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C., and Marone, G., Chem Immunol 62: 108-131, 1995) Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has shown to induce activation of human macrophages (Mozzzoni, A., et al. J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G., et al., Int Arch Allergy Immunol, 124, 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois, J., et al., J. Immunol 164: 2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the Immune response. In this case, both opioids caused significant decrease in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS)

Ferrets challenged with this pathogen have been shown to exhibit anorexia and weight loss (up to 23%) dyspnoea, diarrhea, neurological signs with 100% lethality by d7. Maines et al. 2005. J Virology 79(18):11788-11800. If the animal's exhibit signs that they will not survive to the next observation period they will be humanely euthanized. This is done with full realization that validated endpoints of imminent death do not yet exist for the ferret model. We will use out best scientific judgment, to make the call for euthanasia. However, some animals may not survive to the next observation period.

Study 19: 10 Nonhuman Primates (cynomolgus macaques)

This study is classified as a Category E. It will look at the effect of low challenge doses of an infectious organism on morbidity and mortality in Cynomolgus macaques. The results of this study may be used to support future submissions to the FDA under the "Animal Rule" amendment (21 CFR Parts 314 subpart 1 and 601 subpart H) to FDA's new drug and biological products regulations. The "Animal Rule" applies when adequate and well controlled clinical studies in humans cannot be ethically conducted and field efficacy studies in humans are not feasible. The animal endpoints must clearly relate to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity. Therefore, it is necessary to challenge the NHP with a lethal or effective dose of agent and follow the disease process up to death. Furthermore, we need to study the natural progression of the disease process in the absence and presence of the therapeutic agent without the confounding influence of another agent on the pathophysiological process of infection. Also, it is not my intention as the Study Director to allow the animals to die unobserved in their cages. When the animals exhibit signs that they are moribund, they will be humanely euthanized. Any animals found moribund will be euthanized as quickly as possible.

Category E Explanations

Moribund animals are defined as those demonstrating: seizures, severe depression, or coma; respiratory distress or severe dyspnea; persistent recumbency and weakness, unresponsiveness to touch or external stimuli. This is done with full realization that validated endpoints of imminent death do not yet exist in the NHP model. We will use our best scientific judgment, to make the call for euthanasia; however some NHP's may not survive to the next observation period.

Study 20: 23 Ferrets

This is a study to evaluate the efficacy of vaccines against an infectious agent in ferrets. The ferrets will be vaccinated, challenged with the infectious organism and monitored for disease symptoms or protection by the vaccine. Industry standards and FDA directives [21 CFR Part 314 Subpart/ 9New Drugs) and Part 601 Subpart H 9Biological Products)] dictate the need to use moribund disease as the primary endpoint of vaccine efficacy. The illness experienced by animals exposed to the infection must not be treated with analgesics because treatment could alter the immunologic response to infection and vaccine, thus making it impossible to interpret the data obtained in this study.

When the animals exhibit signs that they will not survive to the next observation period they will be humanely euthanized. This is done with full realization that validated endpoints of imminent death do not yet exist in this ferret disease model. We will use out best scientific judgment to make the call for euthanasia; however, some ferrets may not survive to the next observation period.

Study 21: 22 Ferrets

The objective of this study is to assess the pathogenesis of infection via different routes of administration, as well as to optimize an aerosol delivery model for the future administration of therapeutic in this animal model. Animals being challenged with this pathogen are expect to experience illness related to the infection, including fever, gastrointestinal and neurological disorders, anorexia, dehydration, hematological and clinical chemistry changes, ultimately ending in humane termination (euthanasia) prior to death. Animals being used to optimize the aerosol delivery system are not expected to experience pain or discomfort, and will be anesthetized during these procedures. Illness experienced by challenged animals must not be treated with analgesics, as this would comprise the scientific integrity of the study, mask the pathogenesis of the disease (and obscure secondary efficacy parameters, such as amelioration of clinical signs), could inadvertently accelerate the disease process (additive effect to the infection) and confound the interpretation of euthanasia criteria. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics were shown to interfere with the mechanisms responsible for interferon production. (Geher, W.F. et.al. J. Toxicol Environ Health 2:577-582, 1977: Hung, C. Y., et al. Proc Soc Exp Biol Med 142:106-111-1973). Moreover opioids can suppress Natural Killer (NK) cell activity (Beilin, B., et al. Brain Behav Immun 3: 129-137, 1989). Also, analgesics including buprenorphine can cause histamine release (Marone, G., et al Int Arch Allergy Immunol 124-249-252, 2001: Stellato, C., and Marone, G., Chem Immunol 62: 108-131, 1995) Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has shown to induce activation of human macrophages (Mozzzoni, A., et al. J Immunol 170: 2269-2273, 1999) inhibit interferon alpha release from dendritic cells (Marone, G., et al., Int Arch Allergy Immunol, 124, 249-252, 2001) and increase the synthesis and release of Il-10 from human macrophages (Sirois, J., et al., J. Immunol 164: 2964-2970, 2000). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersman et al, (Lab Anim 33: 328-333, 1999) provide an additional example of how analgesics may modify the expression of the disease process. These investigators, using an established murine model of

Category E Explanations

endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both opioids caused significant decrease in circulating levels of tumor necrosis factor alpha following administration of lipopolysaccharide (LPS).